RECEIVED
CENTRAL FAX CENTER
JAN 11 2007

REMARKS

Amendments

New claims 13 and 26 are directed to the subject matter of canceled claims 11 and 12, respectively, revised to comport with the language of claims issued in US Pat Nos. 6,287,556 and 6,599,502. These amendments introduce no new matter.

More particularly, new claims 13-25 impose the same limitations as claims 30-42 of US Pat No. 6,287,556 except for the preamble, recited cell ("eukaryotic" is narrowed to "human"), and final wherein clause. In particular, new claim 13 is obtained from claim 30 of US Pat No. 6,287,556 as follows:

> A method of generating an immune response comprising the step of introducing a foreign antigenic agent into a ~~eukaryotic~~ cell of a human ~~comprising the step of~~ by contacting the cell with a non-virulent bacterium comprising a first gene encoding a nonsecreted foreign functional cytolysin operably linked to a heterologous promoter which expresses the cytolysin in the bacterium, and a second gene encoding the foreign antigenic agent under conditions whereby the agent enters the cell, wherein an immune response to the agent is generated.

Analogously, new claims 26-43 impose the same limitations as claims 1-18 of US Pat No. 6,599,502 except for the preamble, recited cell ("eukaryotic" is narrowed to "human"), and final wherein clause. In particular, new claim 26 is obtained from claim 1 of US Pat No. 6,599,502 as follows

> A method of generating a physiological response comprising the step of introducing a foreign therapeutic agent into a ~~eukaryotic~~ cell of a human ~~comprising the step of~~ by contacting the cell with a nonvirulent bacterium comprising a first gene encoding a nonsecreted foreign functional cytolysin operably linked to a heterologous promoter which expresses the cytolysin in the bacterium, and a second gene encoding a foreign therapeutic agent, different than the cytolysin, under conditions whereby the ~~therapeutic~~ agent enters the cell, wherein a physiological response to the agent is generated.

Specification Informality

The informality at p.15, lines 12-15 has been corrected as requested.

35USC112, first paragraph (enablement)

The enablement requirement provides that the specification enable one of ordinary skill in the art to practice the invention without undue experimentation. Our claims are directed to a

method comprising the step of introducing a foreign antigenic (claim 13) or therapeutic (claim 26) agent into a human cell by contacting the cell with a non-virulent bacterium comprising a first gene encoding a nonsecreted foreign functional cytolysin operably linked to a heterologous promoter which expresses the cytolysin in the bacterium, and a second gene encoding the foreign agent under conditions whereby the agent enters the cell, wherein an immune (claim 13) or physiological response (claim 26) is generated.

The Specification teaches the introduction of a variety of foreign agents for a variety of purposes (e.g. p.4, line 19 - p.6, line 3), including antigenic and therapeutic agents. The Specification teaches use of a variety of nonvirulent bacteria (e.g. p.6, lines 4-20) and target cells (e.g. p.6, line 21 – p.7, line 12). The specification teaches a variety of effective routes of in vivo and ex vivo administration depending on the nature of the foreign agent (e.g. p.7, line 13 - p.8, line 14). In addition, the specification provides numerous demonstrative examples of the method as applied to numerous foreign therapeutic agents (note that in some embodiments, an antigenic agent will suffice as a therapeutic agent; therapeutic agents include prophylactics such as immunizations, e.g. p.4, lines 21-22) and a variety of target cells in vivo and ex vivo (e.g. p.8, line 15 - p.10, line 11).

The application provides ample teaching to enable one of ordinary skill in this art to practice the claimed method without undue experimentation. In fact, following the teachings of this disclosure without resorting to any undue experimentation, persons skilled in the art have implemented the claimed method to generate an anti-tumour response and to deliver antigens in vivo (e.g. Radford et al., Gene Therapy 2002, 9, 1455-63; Bouwer et al. PNAS 2006, 103, 5102-7; both attached).

For good measure, we provide herewith affirmative evidence in the form of an expert declaration averring to the foregoing. Accordingly, the uncontroverted evidence of record demonstrates that the application provides ample teaching to enable one of ordinary skill in this art to practice the claimed method without undue experimentation.

35USC102(e)

The cited Darji et al. (Cell 1997, 91, 765-75) is neither a patent nor an application for patent, so it is not prior art under 35USC102(e). In any event, the cited publication neither anticipates nor suggests the claimed invention.

Our claims are directed to a method comprising the step of introducing a foreign antigenic (claim 13) or therapeutic (claim 26) agent into a human cell by contacting the cell with a non-virulent bacterium comprising a first gene encoding a nonsecreted foreign functional cytolysin operably linked to a heterologous promoter which expresses the cytolysin in the bacterium, and a second gene encoding the foreign agent under conditions whereby the agent enters the cell, wherein an immune (claim 13) or physiological response (claim 26) is generated.

Darji et al. describe use of modified bacteria as a vehicle for oral genetic immunization. In particular, mice were orally immunized with attenuated *typhimurium aroaA* transformed with eukaryotic expression vectors encoding truncated variants of ActA and listeriolysin (two virulence factors of Listeria monocytogenes) Darji, Abstract. The listeriolysin immunogen was a nonsecreted and nonhemolytic fragment consisting of amino acids 26-482 (e.g. Darji, p.766, col.1, lines 27-29) of the native 529 amino acids (e.g. Lety et al., Microbiology 149 (2003), 1249-1255, attached)

All our claims require a promoter which expresses the cytolysin in the bacterium. Darji et al. teach the opposite: that the cytolysin is expressed not by the bacterium, but by the targeted eukaryotic cell (e.g. Darji, p.766, col.1, lines 5-7, 20-21 and 41-51). Darji et al. require and exclusively teach a eukaryotic promoter, and can not and do not meet the express limitations of our claim that requires a promoter which expresses the cytolysin in the bacterium.

Furthermore, all our claims require expression of a functional cytolysin. Darji et al. teach the opposite: their listeriolysin variant is non-functional (non-lytic), double-truncation (e.g. Darji, p.766, col.1, lines 27-29) – and necessarily so, because their protein is used not for delivery (it is not even expressed until after integration into the eukaryotic cell), but as an immunogen (e.g. Darji, p.766, col.1, lines 23-31; p.768, para. bridging cols.1-2; Fig.4).

35USC103(a)

The cited references (Powell et al., US Pat No.5,877,159, in view of Darji et al. (1995, J Biotech 43, 205-212) and Dietrich et al., 1998, Nat Biotech 16, 181-85) do not suggest the claimed invention.

Our claims are directed to a method comprising the step of introducing a foreign antigenic (claim 13) or therapeutic (claim 26) agent into a human cell by contacting the cell with a non-virulent bacterium comprising a first gene encoding a nonsecreted foreign functional

cytolysin operably linked to a heterologous promoter which expresses the cytolysin in the bacterium, and a second gene encoding the foreign agent under conditions whereby the agent enters the cell, wherein an immune (claim 13) or physiological response (claim 26) is generated.

Powell et al. use live, invasive bacteria to enter animal cells and deliver eukaryotic expression cassettes for expression in said animal cells (col.6, lines 48-62). In addition to naturally invasive bacteria, Powell et al. propose that noninvasive bacteria could be genetically engineered to enter the cytoplasm of animal cells, using various invasive proteins, including listeriolysin O of Listeria (col.8, lines 36-42; col.10, line 35 – col.11, line 11). Powell et al. require and exclusively teach that live bacteria enter the cytosol or nucleus of the target animal cell (e.g. col.8, lines 36-42; col.10, lines 45-46 and 50-51; line 11; col.13, lines 64-65; col.14, liens 5-6 and 14-15).

Darji et al. (1995) teach a method for hyper-expressing listeriolysin in the nonpathogenic *Listeria innocua* for purification (e.g. Abstract; p.206, col.2, lines 25-32). The method provides a high yield of *secreted* listeriolysin (e.g. Results, Section 3.1.)[1]

Unless setting out to use our disclosure as a template to reconstruct our invention, it is not clear how one of skill in the art would be motivated to supplement Powell with Darji. Powell teaches how to deliver and express eukaryotic expression vectors into animal cells, and Darji (1995) teaches how to make and purify large amounts of listeriolysin from a bacterium. The methods of Powell have no use for large amounts of purified listeriolysin.

Dietrich et al. describe delivery of antigen-encoding plasmid DNA into the cytosol of macrophages by attenuated suicide Listeria monocytogenes. Dietrich's method uses a Listeria monocytogenes bacterium having attenuated virulence and expressing a bacteriophage lysis gene (PLY118 – the suicide gene) under the L. monocytogenes *actA* promoter, which is activated in the cytosol of the infected cell; Dietrich, p.181, col.2, lines 17-18. Dietrich's bacteria are specifically designed to escape to the cytosol of the infected cell where the suicide gene can be activated; Dietrich, p.181, col.1, line 24; p.184, col.2, lines 7-9. The antigens (GFP, CAT

---

[1] Unlike the relied-upon Darji (1995), Darji (1997) does describe a non-secreted listeriolysin protein immunogen; however, as noted above, that doubly-truncated immunogen is necessarily a nonfunctional lysin.

(chloramphenicol acetyl transferase), and OVA257-264 (T cell-reactive H-2K epitope) are then expressed by the host macrophage through a eukaryotic promoter (Dietrich, p.183, col.1-2).

The Action proposes imposing Dietrich's PLY118 gene[2] to "add to the safety of the attenuated L. monocytogenes".... But Powell et al. use live, invasive bacteria to enter animal cells and deliver eukaryotic expression cassettes for expression in said animal cells (supra), so adding a PLY118 gene would only yield the method of Dietrich.

The cited references have not been shown to provide any suggestion for introducing a foreign agent into a human cell using non-virulent bacterium comprising a first gene encoding a nonsecreted foreign functional cytolysin operably linked to a heterologous promoter which expresses the cytolysin in the bacterium, and a second gene encoding the foreign agent under conditions whereby the agent enters the cell.

---

[2] PLY118 is not even a cytolysin – it does not lyse any cellular membranes. It is a peptidase endolysin – it is secreted through the membrane of the bacteria and attacks a component of the surrounding bacterial wall; see Dietrich et al. p.182, col.1, line 1, where Dietrich cites Loessner et al., 1995, Mol Microbiol 16, 1231-41, abstract enclosed. Loessner explains that PLY118 is a cell wall lytic enzyme which specifically cleaves between the L-alanine and D-glutamate residues of listerial peptidoglycan.

The Examiner is invited to call the undersigned with any suggestions for amending the claims or further clarifying any of the foregoing. Please charge any required fees to our Dep. Acct. No.19-0750 (order B98-039-4).

Respectfully submitted,
Science & Technology Law Group

Richard Aron Osman, J.D., Ph.D., Reg. No. 36,627
Tel (949) 218-1757; Fax (949) 218-1767

Encl. Lety et al., Microbiology 149 (2003), 1249-1255
Loessner et al., 1995, Mol Microbiol 16, 1231-41, abstract
Radford et al., Gene Therapy 2002, 9, 1455-63
Bouwer et al. PNAS 2006, 103, 5102-7
Declaration under 37CFR1.132
PTO/SB/08B
PTO-2038 for Extension (3mos.), Claims (11)

"To Help Our Customers Get Patents"
Mission Statement, USPTO External Customer Services Guide